

SHELL BANDING POLYMORPHISM IN *CEPAEA VINDOBONENSIS* IN RELATION TO HABITAT IN SOUTHEASTERN POLAND

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ABSTRACT

We studied the frequencies of shell-banding variants of *Cepaea vindobonensis* (Férussac) in open, semi-open and shaded habitats in two geographically separate but climatically similar areas in southeastern Poland. The faint-banded phenotype was more frequent in open and semi-open habitats than in shaded areas. Within the dark-banded phenotype, those with missing bands were associated with open and semi-open habitats, and those with fusion of bands with shaded habitats. The feature most strongly associated with habitat type was the width of bands relative to the ground area. It was significantly higher in shaded habitats than in semi-open areas, in which it was significantly higher than in open habitats. Our study thus indicated the effect of local microclimatic selection on the frequencies of various phenotypes, resulting in light shells being more common in open habitats and dark shells in shaded areas. This association of shell characteristics with habitat type was more pronounced in the area where *Cepaea vindobonensis* inhabited more natural habitats and showed higher polymorphism.

Key words: color, climatic selection, land-snails, microclimate, natural selection, pigmentation, rate of evolution, *Cepaea*.

INTRODUCTION

Cepaea vindobonensis (Férussac, 1821) is a helicid land snail inhabiting southeastern Europe; its continuous distribution ranges from the northern Caucasus to the Balkans, Appenine Peninsula, eastern Austria, Czech Republic, and southeastern Poland (Kerney et al., 1983; Riedel, 1988). It is a xerothermophilic species, usually inhabiting open and exposed habitats. In the central and southern parts of its distribution it also occurs in woods and other tree-covered sites (Rotarides, 1926; Jones, 1975; Dhora, 1985; Kramarenko et al., 2007). Polymorphism in this species differs from the extensive polymorphism in the congeners *Cepaea nemoralis* (Linnaeus, 1758) and *Cepaea hortensis* (Müller, 1774) in that the ground color of the shell is always yellowish to white, and the usual number of bands in most populations is five. Bands may be missing or fused in some populations. Two major forms can be distinguished: dark-banded and faint-banded, with dark-brown-colored and straw-colored bands, respectively. In *C. nemoralis* and *C. hortensis*, the ground color of the shell, presence or absence of bands, number of bands on a banded shell and fusion of bands are

genetically controlled (Jones et al., 1977). No data on the formal genetics of *C. vindobonensis* are available, but by analogy to other *Cepaea* species genetic control of the shell banding polymorphism may be assumed. There are only a few studies relating shell polymorphism to environmental factors in *C. vindobonensis*; most suggest an effect of climate. In the Czech Republic (Honěk, 2003), the faint-banded morph occurs almost exclusively south of the 17°C isotherm of mean June temperature, and the increase in frequency of dark-banded morph from south to north parallels the decrease in average April–August temperature and sunshine hours. Also, populations in open, grassy habitats tend to include pale morphs, and these are at higher frequencies than in dense forb stands (Honěk, 2003). In Greece, in a hot and dry area near the Axios River, the faint-banded morph reaches 30%, in contrast to the cooler and more humid region of Logos, where it is extremely rare (Staikou, 1999). In Croatia, morph frequencies are associated with topography, which can be readily explained by climatic selection (Jones, 1973, 1974).

In these previous studies, variation was analyzed only as faint-banded and dark-banded. However, within the typical five-banded form,

the width of individual bands can vary considerably, resulting in variation in the proportion of the shell covered with bands, which can be compared among populations. Such an approach was first used in the largely forgotten study of Rotarides (1926); we also used it here.

In *Cepaea*, climatic selection can affect morph frequencies on a large geographic scale (Jones et al., 1977) and on the scale of habitats (OŹgo, 2005). In this study, we related the polymorphism of *C. vindobonensis* to habitat type in two geo-

graphically distant but climatically similar areas in southeastern Poland. This allowed us to study the effect of similar selective forces on populations influenced by different historical factors.

MATERIAL AND METHODS

The study was carried out in two areas in southeastern Poland: the Hrubieszów area and Sandomierz area (Fig. 1), both at ap-

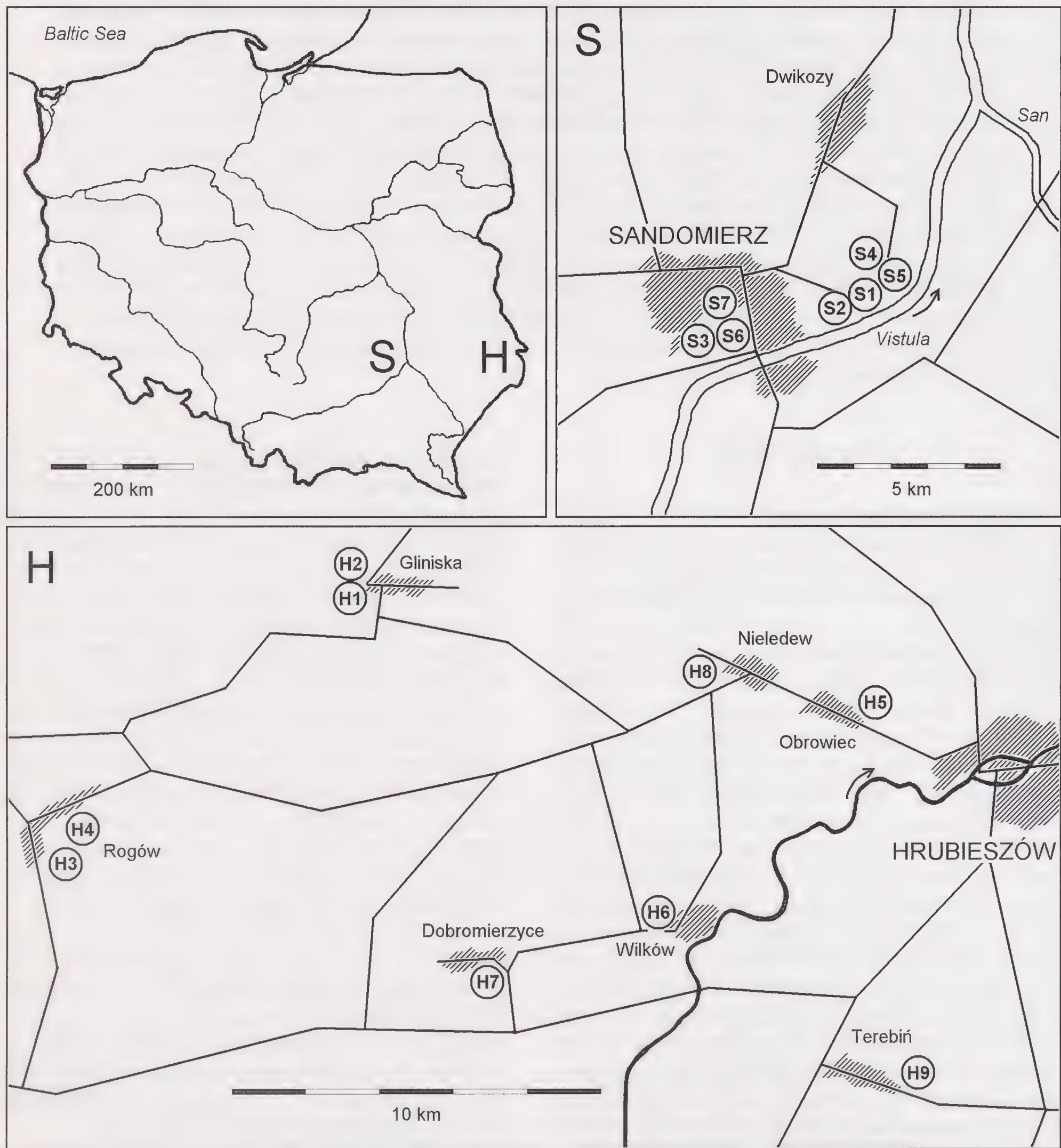


FIG. 1. Maps of Poland showing the location of the study sites. H1–H9, populations in the Hrubieszów area; S1–S7, populations in the Sandomierz area.

proximately 50°N and 200 m a.s.l, but 140 km apart. Climatic characteristics of the areas are similar: in the Hrubieszów area, average annual temperature is 7.2°C, average May–September temperature is 15.2°C, and average July temperature is 17.4°C; annual precipitation is 563 mm, and precipitation in May–September is 340 mm. In the Sandomierz area these values are 7.6°C, 15.6°C and 17.8°C, 568 mm and 339 mm, respectively (data for 1961–1990 from the Institute of Meteorology and Water Management, Kraków). In the Hrubieszów area, the snails occurred in fairly stable populations inhabiting remnants of natural habitats, whereas in the Sandomierz area they occurred mostly in more disturbed semi-natural habitats in urban and suburban environments.

We collected samples in 2002–2005 from areas of uniform vegetation. The collection sites were as small as practically possible and usually did not exceed 1,000 m². We collected live snails and well-preserved dead shells, and only mature individuals with a well-developed lip. We included only sites where we obtained samples of 30 or more, for a total of 1,032 individuals from 16 populations. Populations of adequate size were hard to find.

We divided the habitats into three categories: open, semi-open, shaded. Open habitats included escarpments covered with short

vegetation and xerothermic meadows. Shaded habitats were those with trees dense enough to give shade throughout the day. Semi-open sites were intermediate in character, with shrubs or scattered trees; places where snails occurred only at the edge of a wooded area were included in this category.

We first scored the shells as faint-banded or dark-banded. Bands in the faint-banded morph are often blurred and spread into areas of whitish coloration, giving the shell an overall yellow appearance; we therefore did not score these shells further. Within the dark-banded phenotype, we scored missing and fused bands, and we estimated the proportion of the area of the shells covered with bands. Within dark-banded shells with all bands present and separate, we estimated the relative width of consecutive bands (Rotarides, 1926). First, we measured the width of the bands and of the ground area to an accuracy of 0.5 mm, and calculated the percentage of the shell covered with bands (%B). We took the measurements at a line across the body-whorl at right angles to the lower lip of the mouth, as is standard in scoring band fusion in *Cepaea* species (Cain & Sheppard, 1950). As noted by Rotarides (1926), the accuracy of 0.5 mm is optimal; greater accuracy would not improve the results because of the frequent smudging and spreading of the bands. Second,

TABLE 1. Composition of *C. vindobonensis* samples. Missing bands – number of shells with any bands missing in the dark-banded phenotype; Fused bands – number of shells with any bands fused in the dark-banded phenotype; Average %B – average proportion of the area of the shells covered with bands in the dark-banded phenotype. Habitats: O – open; I – intermediate; S – shaded.

Sample number	Total snails	Faint banded	Dark banded	Missing bands	Fused bands	Average %B	Habitat
H1	98	76	22	6	0	29.4	O
H2	67	23	44	7	1	31.4	I
H3	71	0	71	3	0	34.9	S
H4	51	1	50	1	0	35.3	O
H5	49	10	39	0	0	36.5	O
H6	100	9	91	1	4	37.5	O
H7	79	0	79	0	16	48.1	S
H8	63	5	58	0	15	50.2	S
H9	57	12	45	0	18	50.6	S
S1	46	7	39	0	0	37.6	O
S2	56	6	50	0	0	39.1	O
S3	98	2	96	3	0	41.6	O
S4	31	0	31	0	0	42.8	I
S5	30	8	22	0	0	45.1	I
S6	65	0	65	0	1	46.1	S
S7	71	1	70	0	0	46.7	S

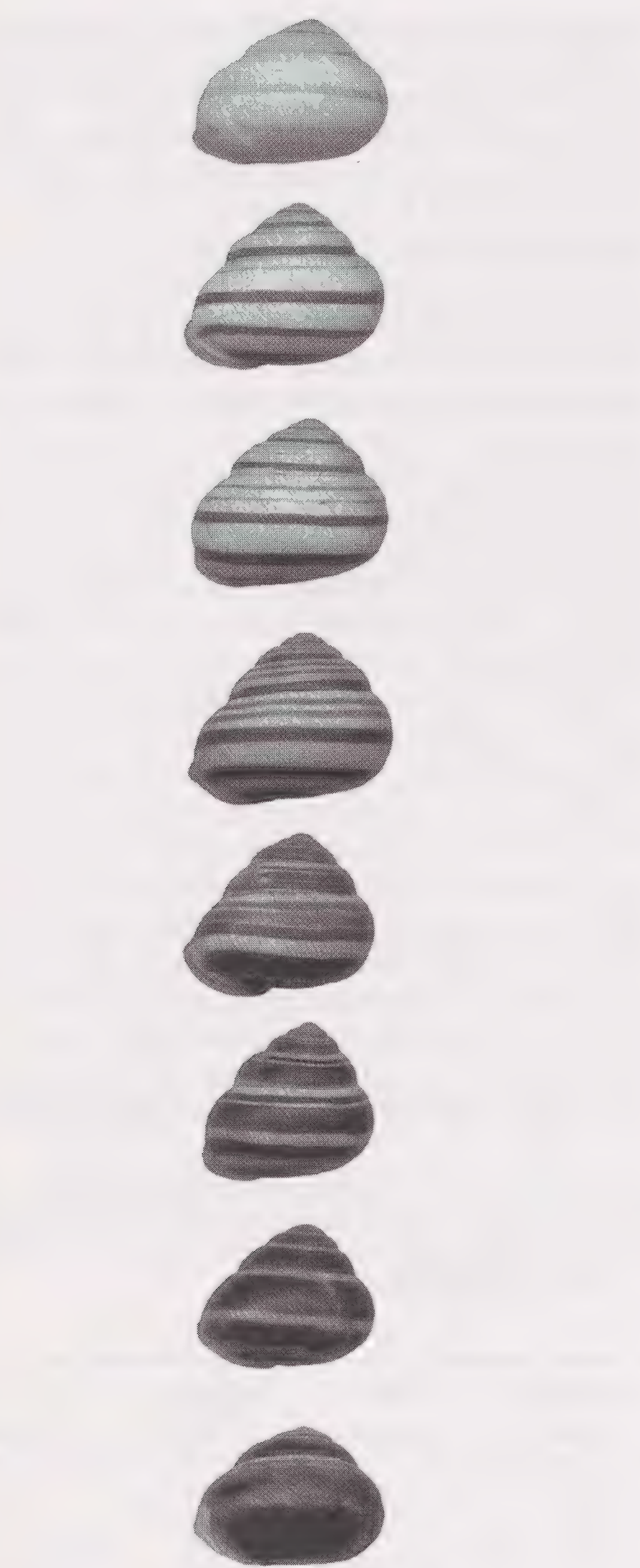


FIG. 2. The range of variation of *C. vindobonensis* shells observed in this study. From top to bottom: faint-banded shell; dark-banded shell with band 2 missing - 10345; dark-banded shell with very narrow bands - 1 = 2 < 3 = 4 < 5; dark banded shell with bands of medium width - 1 = 2 < 3 = 4 = 5; dark-banded shell with wide bands - 1 < 2 > 3 < 4 > 5; dark-banded shell with fused bands - 1(23)45; dark-banded shell with fused bands - (123)45; dark-banded shell with fused bands - (12345).

we compared the width of each band to that of its neighbors; 1 = 2 = 3 = 4 = 5 designates a shell with equal width of all bands, while 1 < 2 > 3 = 4 = 5 indicates that the first band is nar-

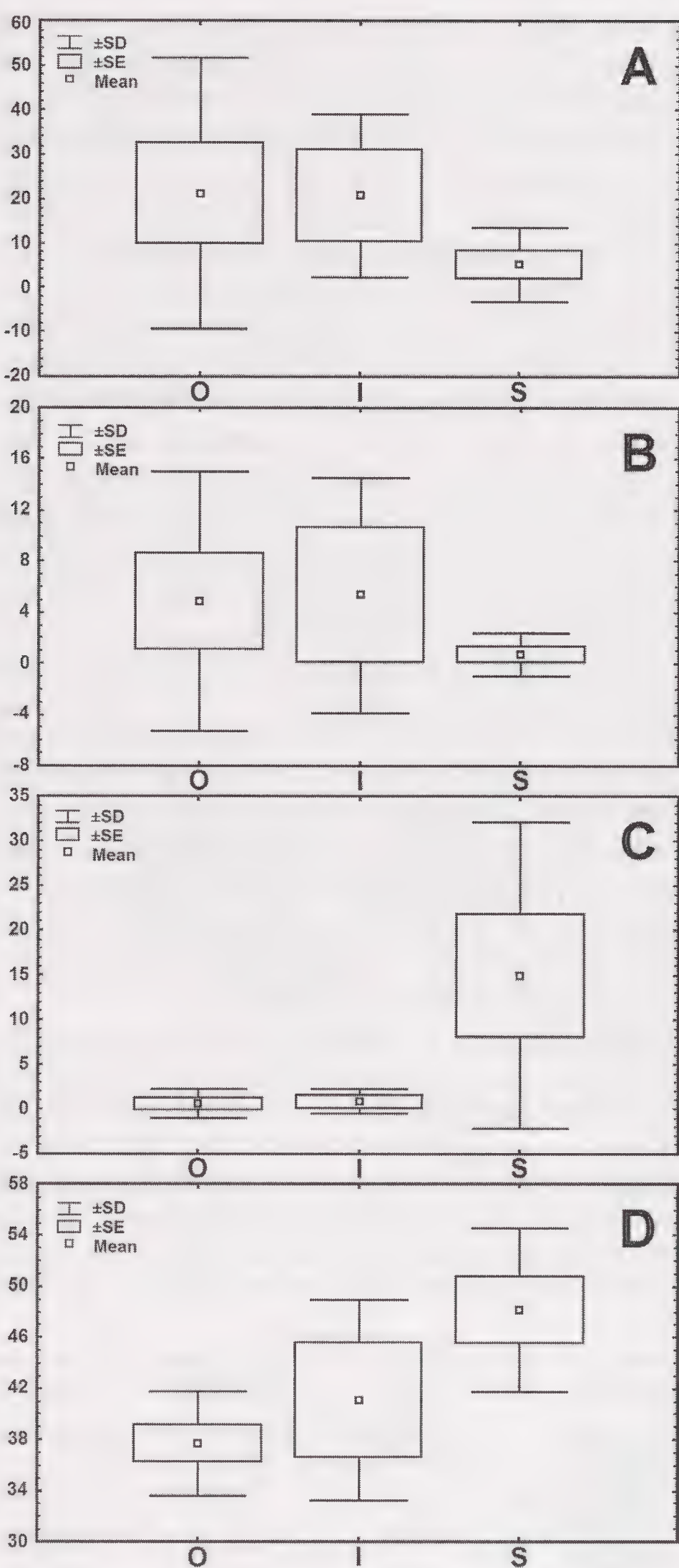


FIG. 3. Association of the frequencies (%) of the major *C. vindobonensis* morphs with habitat. A – faint-banded shells, B – shells with missing bands, C – shells with fused bands, D – the proportion of shells covered with bands (%B); O, open habitats n = 7; I, intermediate habitats n = 3; S, shaded habitats n = 6; SD, standard deviations; SE, standard errors. Percentage values arcsin transformed.

rower than the second, which is wider than the third; theoretically in shells with all five bands present and separate, there are 81 possible patterns (Rotarides, 1926).

RESULTS

The composition of the samples is given in Table 1. Figure 2 illustrates the observed range of variation of *C. vindobonensis*, and Figure 3 shows variation in frequencies of shell variants in each habitat type. The faint-banded phenotype occurred in both study areas and in most populations. It was more frequent in open and intermediate habitats than in shaded areas, but the difference just failed to reach significance (Mann-Whitney test, $p = 0.056$). Shells with fused or missing bands within the dark-banded phenotype were much rarer than faint-banded shells and occurred almost exclusively in the Hrubieszów area. Shells with missing bands were associated with open and intermediate habitats, in which 86% of them occurred (sites H1, H2, H4, H6, and S3), while only three individuals came from a shaded habitat (H3 – but see Discussion). Differences in frequency among habitat types were, however, not significant, probably because of the small overall number of such shells ($n = 21$). Shells with fused bands occurred mostly (91%) in shaded habitats (sites H7, H8, H9, and S6), while only four individuals came from an open habitat (H6) and one individual from an intermediate habitat population (H2). The difference in frequency between shaded and other habitat types was statistically significant (Mann-Whitney test,

$p = 0.046$). Thus, although only one of those three variants shows a formally significant relationship to habitat, all three show the trend expected from earlier work, both in median values and in the proportion of sites in which the variants occur.

The width of the bands relative to the ground area, that is, the proportion of the shells covered with bands (%B), showed the strongest association with habitat type. The average %B was higher in shaded than in intermediate habitats, in which it was higher than in open areas (Fig. 3 D), (Kruskal-Wallis test, $p = 0.049$).

In Figure 4 sites are arranged in order of increasing average %B. Significance of differences among the sites was established with the nonparametric multiple comparisons test with unequal sample sizes (Zar, 1999). Populations in group 1 do not differ significantly from each other, but all are significantly different from those in group 2, which again do not differ significantly amongst themselves. In the mid range (S1 to S4 inclusive), S2 and S3 differ from both extremes, but not from all members of either group; S1 and S4 differ from one or the other extreme, but not from all members of the group to which it belongs. Most shaded habitats belong to group 2, confirming the result above: differences between shaded habitat populations of group 2 and every population of group 1 are all highly significant ($p < 0.001$).

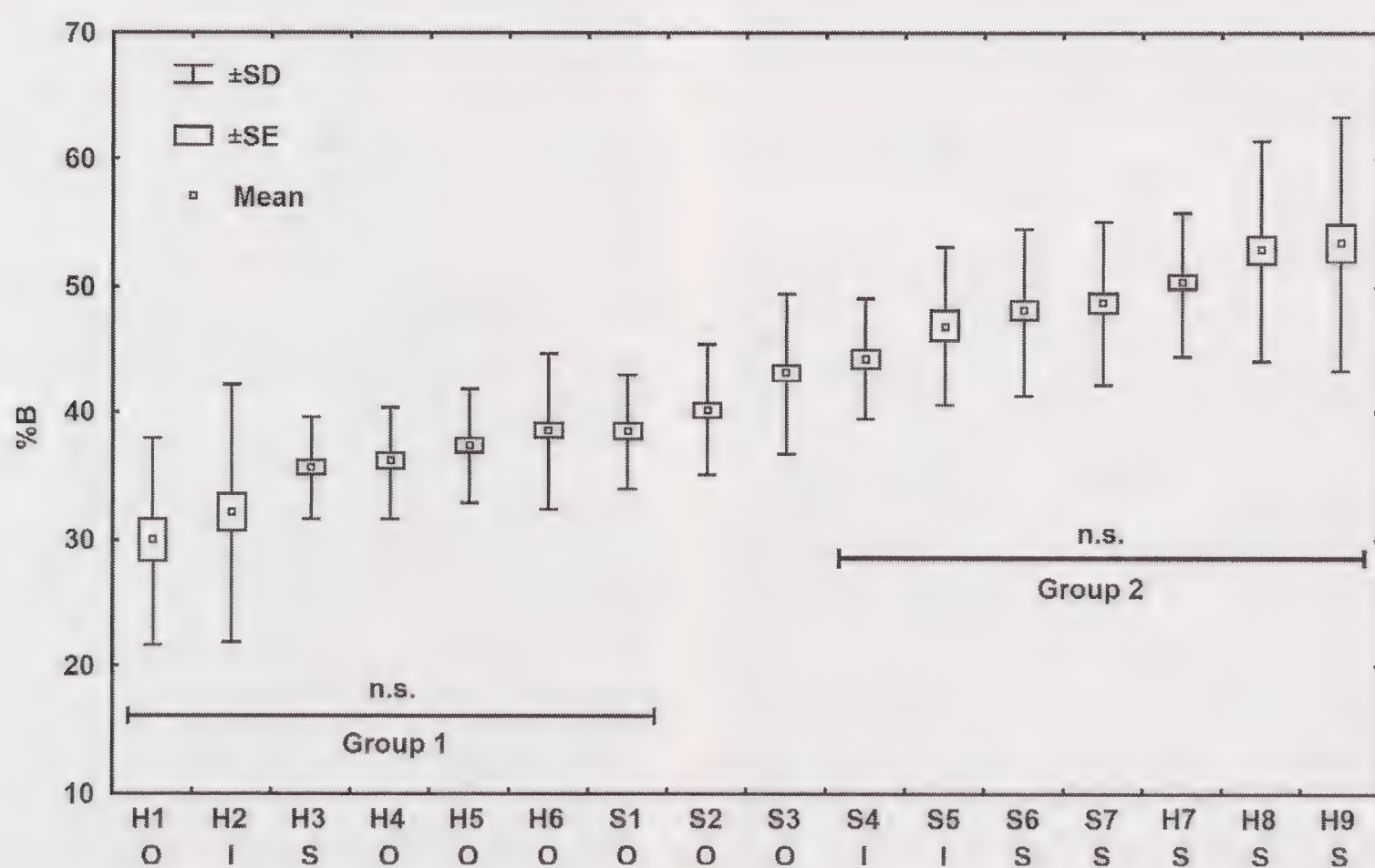


FIG. 4. Mean values, standard deviations (SD), and standard errors (SE) of the proportions of shells covered with bands (%B) in *Cepaea vindobonensis* populations, percentage values arcsin transformed. H1–H9, populations in the Hrubieszów area; S1–S7, populations in the Sandomierz area; O, open habitats; I, intermediate habitats; S, shaded habitats; n.s., no statistical significance in the nonparametric multiple comparisons test.

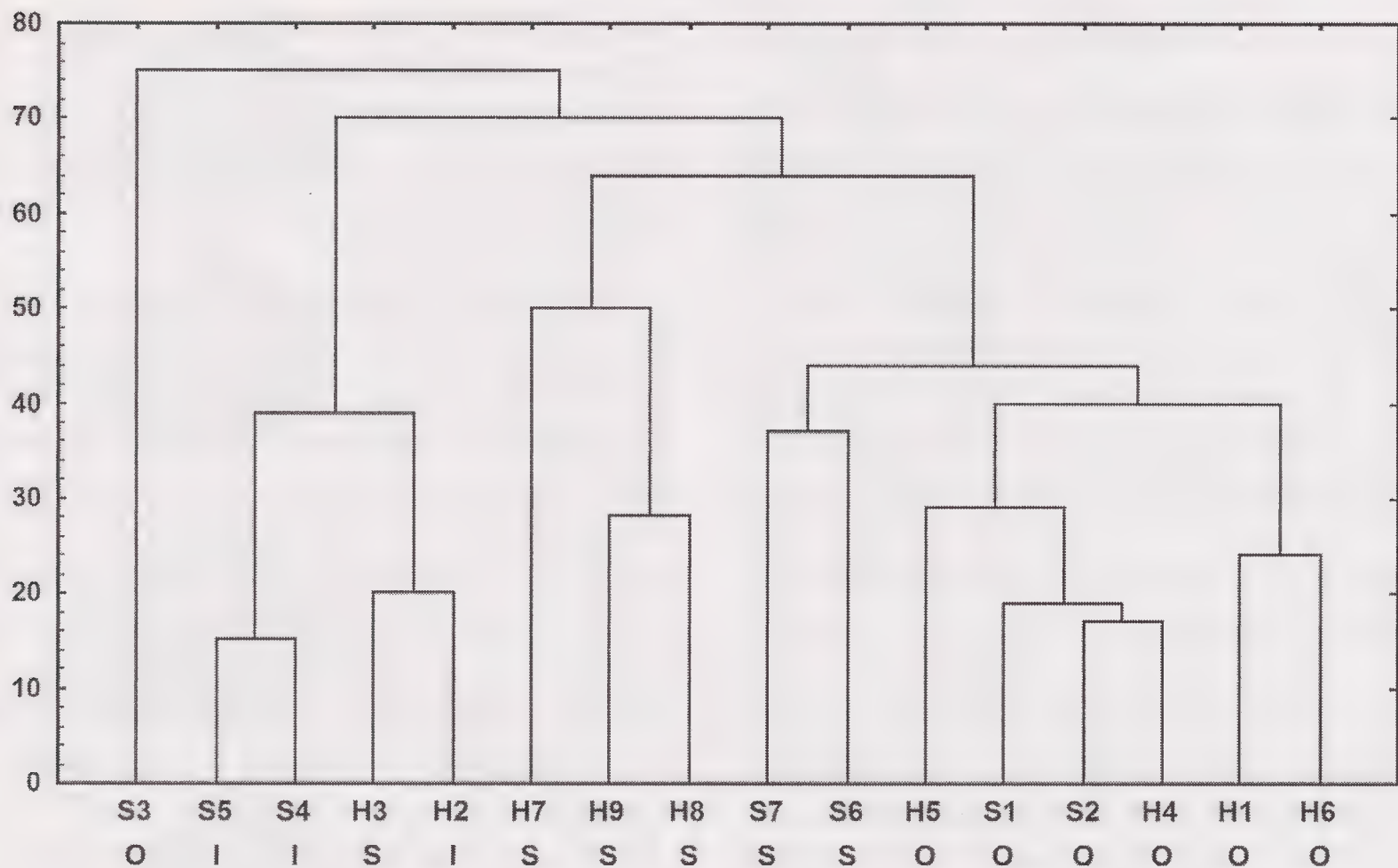


FIG. 5. Dendrogram of percentage of similarity affinities between sites. H1–H9, populations in the Hrubieszów area; S1–S7, populations in the Sandomierz area; O, open habitats; I, intermediate habitats; S, shaded habitats.

Populations in the Hrubieszów area exhibit more extreme values of %B than populations in the Sandomierz area, which group together in the middle of the graph. The anomalous position of H3 is considered below.

The width of consecutive bands on a shell revealed 25 banding variants. The two commonest ($1 = 2 < 3 < 4 < 5$ and $1 = 2 < 3 = 4 < 5$) occurred at all sites reaching an overall frequency of approximately 30% each, and within each there was the same trend for a greater percentage of band cover in shaded habitats (data not shown).

Frequencies of other variants varied considerably among populations, and all variation has been used to examine the similarity in occurrence of the 25 variants of relative band thickness among sites using the percentage of similarity (Southwood, 1968). Figure 5 shows the dendrogram of affinities among sites. Populations from open and intermediate habitats cluster together with only one "mismatch" (site H3 – but see Discussion). Within these clusters populations from both study areas are present. Populations from shaded habitats also cluster together, but form two groups connected at a low affinity level; those groups are consistent with geographical location. One open habitat population (S3), geographically close to S6 and S7, is very distinct and does not cluster with any of the rest.

DISCUSSION

Shell variation in *C. vindobonensis* is mainly in band pigmentation and cover. Some of this variation is continuous; its heredity is unknown, and the term polymorphism is used only for convenience. Variation in the darkness of the shells results from the color of the bands, and in the dark-banded phenotype from the number of missing or fused bands and from their width. Although the small number of samples available has restricted our ability to detect significant differences between habitats, the results of our study show that light shells were more common in open habitats and dark shells in shaded areas, which is consistent with the predictions of climatic selection.

Dark-banded *C. vindobonensis* placed experimentally in direct sunlight reach a significantly higher equilibrium temperature than faint-banded individuals of the same size (Jones, 1973; Stai-kou, 1999). In *Cepaea nemoralis*, five-banded phenotypes, especially those with fused bands, reach significantly higher internal temperatures (Heath, 1975), reflect significantly less light, lose more weight, cease activity earlier (Chang, 1991), and suffer more from differential mortality in direct sunshine than the unbanded phenotypes (Richardson, 1974). Unbanded shells are extremely rare in *C. vindobonensis*, but within the five-banded phenotype, the width of bands varies

considerably resulting in large variation in the general darkness of the shells. This presumably affects such features as spectral reflectance, internal temperatures, weight loss and activity. In *Helicella candicans* (Pfeiffer, 1841) (synonyms: *Helicella obvia*, *Xerolenta obvia*), which exhibits similar polymorphism to that in *C. vindobonensis*, shells with wide and fused bands have a higher rate of heating and reach equilibrium temperatures up to 2.5°C higher than shells with narrow bands (Honěk, 1993). In this species, dark shells are more common in areas with frequent fogs and greater cloudiness, and shells in habitats with dense and tall vegetation tend to be darker than those in short grass steppes (Honěk, 1993). In polymorphic populations of *C. nemoralis*, climatic selection affects mostly the ground color of the shells (Jones, 1973; Ožgo, 2005). However, in introduced populations with only yellow shells, it is the presence and fusion of bands that respond to local climatic selection: shadier habitats have higher proportions of banded shells and band fusions than sunnier ones (Richards & Murray, 1974). In this case, the polymorphism of *C. nemoralis* is analogous to that of *C. vindobonensis*, and the response to local climatic variables is similar.

Our results agree with those of Rotarides (1926) from Hungary. He compared two populations of *C. vindobonensis*; in the wetter and cooler wood, the shells were significantly darker than in the warmer and drier area. He gave an adaptive explanation, relating the properties of the shells to the microclimatic characteristics of the habitats. It is worth noting that his paper was published at a time of general consensus that visible variation in *Cepaea* was non-adaptive (Cameron, 1997), and a quarter of a century before the first widely recognized paper on the effect of selection on morph frequencies (Cain & Sheppard, 1950).

In our two study areas, the association of the proportion of shells covered with bands (%B) with habitat shadiness was similar, although more pronounced in the Hrubieszów area (Fig. 4). This may be because populations in the Hrubieszów area were more polymorphic for missing and fused bands – characteristics with which %B is strongly correlated (within the dark-banded category correlation between the frequency of shells with missing/fused bands and average %B in populations is $r = -0.656$, $p < 0.01$, and $r = 0.653$, $p < 0.01$, respectively). Hrubieszów populations inhabit more natural and stable habitats, suggesting a longer time for adaptation. These two explanations may be related to each other: greater variation in Hrubieszów may result from the greater habitat stability. In contrast, however,

urban populations in southern Ukraine are more polymorphic than natural ones (Kramarenko et al., 2007). Differences between the Hrubieszów and Sandomierz areas may also have broader biogeographic causes. There are no major barriers between the Hrubieszów area and Ukraine, where shells with missing or fused bands are relatively common (Sverlova & Kirpan, 2004; Kramarenko et al., 2007). The Sandomierz area is on the other, western side of the Vistula River, a major geographic barrier. In populations of *C. vindobonensis* in western parts of the distribution of the species, missing or fused bands appear to be extremely rare (Honěk, 2003; our own unpublished data); possibly those two areas were colonized from different (eastern and western) refuges in the post-glacial period. It is also possible that the limited variation reflects some kind of founder effect, with Sandomierz populations carrying only a subset of the western range of variation.

The occurrence of the 25 variants of relative band thickness follows a habitat pattern (Fig. 5), but within shaded habitats two distinct geographical groups are present. This suggests some kind of selection. However, the genetic control of minor variants of shell banding is unknown, and there may be complex interactions between relative thickness of different bands and the proportion of the shell covered by them. The different results between areas for shaded habitats suggest an origin effect. As the habitat of *C. vindobonensis* is generally xerothermic, perhaps when confronted with the availability of more shaded habitats, the populations in the two areas increased their overall darkness in different ways, which is possibly just a chance effect.

Three populations need additional comment. The H3 population inhabited a wooded area, but its shell characteristics were typical of open habitats. However, the wood was a fairly recent one, developing after establishment of a nature reserve in 1965. The reserve was meant to protect xerothermophilic flora, but it was not managed and became overgrown with trees. The adaptation of *C. vindobonensis* appears to be much slower than the change in the habitat, and present shell characteristics of this population are possibly one of the few remnants of the original character of this place. This slow rate of adaptation contrasts with introduced *C. nemoralis* populations in southeastern Poland, in which significant shift in the frequencies of color and banding morphs in response to local climatic selection can occur in less than 20 years (Ožgo & Kinnison, 2008). Because the H3 population segregates with intermediate habitat populations in the dendrogram of banding pattern affinities, it

may have already shifted in character towards more shaded habitats.

Populations H1 and H2 inhabited two sides of the same hill: a south-facing slope covered with short grasses (H1) and a north-facing slope with shrubs and sparse trees (H2). The history of this place is unknown, but it is likely that these populations share a common origin. Generally in the area of our study, the feature most strongly associated with habitat type was the proportion of the shells covered with bands (%B) in the dark-banded phenotype. This value was slightly but not significantly higher in the northern (H2) than in the southern (H1) population (Mann-Whitney test, $p > 0.05$). However, these populations differed significantly in the frequency of faint-banded shells: 34% in the northern population, 78% in the southern population ($\chi^2 = 30.98$, $p < 0.001$). In this case, climatic selection apparently affected the frequencies of faint-banded shells, not the width of bands in the dark-banded shells.

Our results show that the *C. vindobonensis* shell polymorphism reacts to local climatic selection but, as exemplified by the H3 population, the adjustment proceeds relatively slowly. The major mechanism of adaptation is through the width of bands in the dark-banded shells, but in some circumstances it is the frequency of faint-banded shells that can be affected. The specific route depends presumably on initial gene-pools and/or the nuances of local selection.

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